Enhancing DNASep® Cartridge Performance and Lifetime by Using Transgenomic Optimase™ Polymerase Buffer

The DNASep® Cartridge gives the WAVE® Nucleic Acid Fragment Analysis System its unparalleled sensitivity in nucleic acid analysis. Maintaining the performance of the DNASep Cartridge requires care in the choice and preparation of reagents and samples used with the WAVE System. Transgenomic Optimase™ Polymerase has been developed to avoid components that are detrimental to the performance of the WAVE System, thereby helping to ensure the highest possible quality of analysis and greatest DNASep Cartridge lifetime.

Introduction

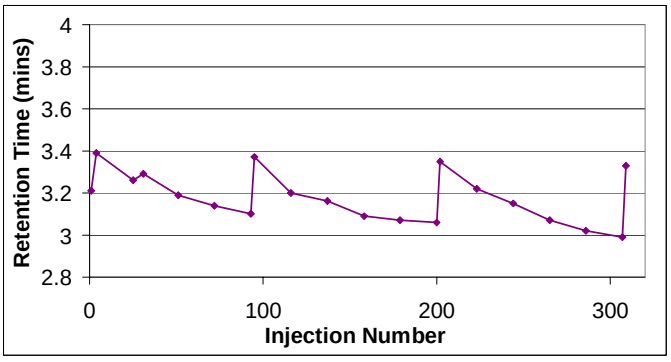
The core technology of the WAVE® Nucleic Acid Fragment Analysis System is the DNASep® Cartridge. Positively charged triethylammonium ions in the buffer solution interact with the negative charges on the DNA backbone giving the DNA molecules a hydrophobic outer coating. The hydrophobic trialkyl groups interact with the hydrophobic surface of the DNASep Cartridge matrix, thereby immobilizing the DNA.

During a sample analysis, the concentration of acetonitrile is gradually increased until these hydrophobic interactions are disrupted and the DNA is eluted from the cartridge. The addition of any component that may interfere with these interactions will have a detrimental effect on the quality and reproducibility of separations achieved using the DNASep Cartridge, and may ultimately shorten its lifetime.

Results and Discussion

Transgenomic has built up considerable information regarding components of polymerase reaction buffers that have a detrimental effect on DNASep Cartridge performance. Some of these components can be removed by following Transgenomic's recommended cleaning procedures (Figure 1a and 1b) while others cause permanent damage (Figure 2). All of these components reduce both DNASep Cartridge lifetime and the quality of analysis that is possible. Most harmful components can be avoided by using appropriate reagents with the WAVE System. It is important therefore to carefully consider the components of any PCR* buffer used in preparing samples for analysis with the WAVE System. The use of a polymerase preparation that is compatible with the WAVE System ensures

1a



1b

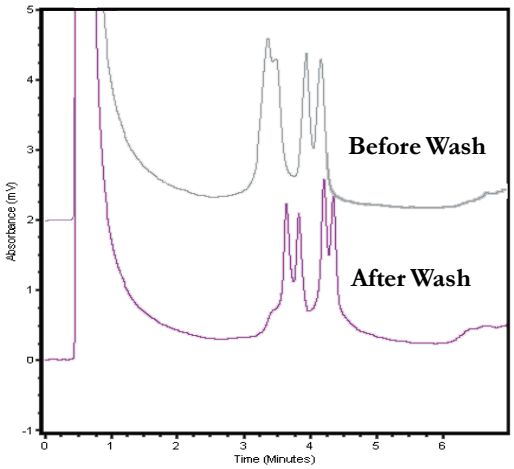


Figure 1: The effect of non-compatible PCR buffers on sample analysis.

A selected polymerase buffer was diluted using HPLC grade water to the concentration specified by its manufacturer. Standard 5µL aliquots were injected onto the DNASep Cartridge and eluted using a linear acetonitrile/0.1 M triethylammonium acetate gradient of 13.75 to 15.5% acetonitrile (55 to 62% buffer B) over 3.5 minutes at 56°C. A mutation standard was run after every 20 injections using a 5µL injection volume and recommended gradient procedures at 56°C. The retention times plotted are taken from the first heteroduplex peak. A cartridge wash procedure using 75% acetonitrile at 70°C was carried out after every 100 injections of polymerase buffer.

(a) Retention time variation using a reaction buffer showing recoverable incompatibility with the DNASep Cartridge.

(b) Changes in the resolution of the WAVE™ Low-Range Mutation Standard before and after cartridge washing.

that results are more consistent, the DNASep Cartridge lasts longer and the need for remedial cleaning procedures is reduced. Figure 3 shows compatibility data from the DNASep Cartridge when used with the Optimase Polymerase buffer system, demonstrating the reproducibility that is possible over extended injection numbers.

In order to investigate the compatibility of PCR products amplified using Optimase Polymerase, a further extended trial was set up to examine retention times over more than 6,000 injections. This showed that under conditions comparable to those found in any standard laboratory, combining the correct polymerase with recommended cleaning procedures can ensure high quality analysis over thousands of injections.

Conclusion

The WAVE® System is often utilized in critical applications where it is essential that the quality of analysis is reproducible over extended periods of time. Many PCR buffers contain components that reduce the quality of analysis that can be achieved through subtle cumulative effects on the binding of DNA to the DNASep Cartridge. The Optimase Polymerase buffer system has been developed with great care to eliminate components that are detrimental to the DNASep Cartridge, ensuring the highest possible quality and reproducibility of analysis.

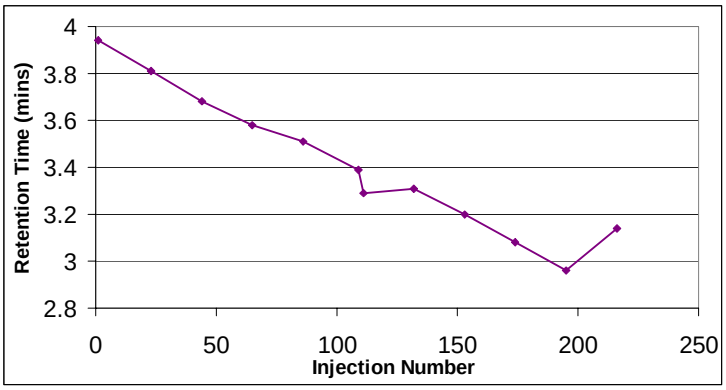


Figure 2: Results from a polymerase buffer having unrecoverable incompatibility with the DNASep Cartridge.
Analysis was carried out as described for Figure 1. No recovery in resolution was observed after washing and the experiment was terminated after 220 injections due to severe cartridge deterioration.

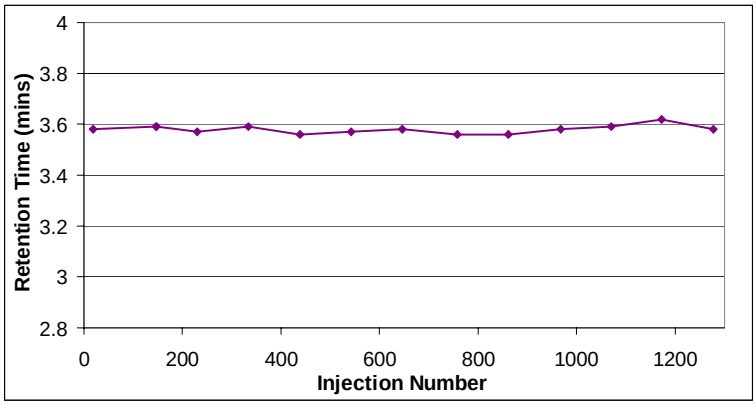


Figure 3: Results for the Optimase Polymerase buffer.
Analysis was carried out over 1200 injections as described for Figure 1. Resolution of the WAVE™ Low-Range Mutation Standard remained unchanged and no significant variation in retention times was observed.

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* PCR is a process covered by patents issued and applicable in certain countries. Transgenomic does not encourage or support the unauthorized or unlicensed use of the PCR process.

Purchase of Optimase™ Polymerase is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process for research applications in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applera or as purchased, i.e., an authorized thermal cycler.

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